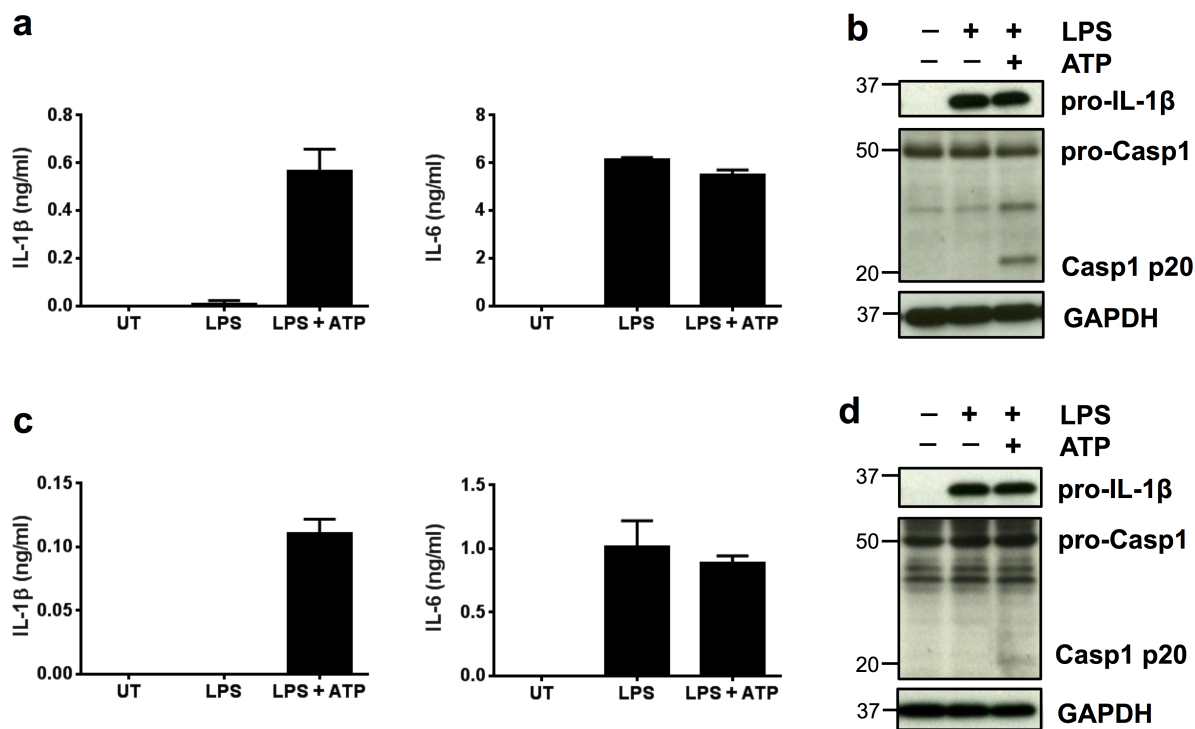
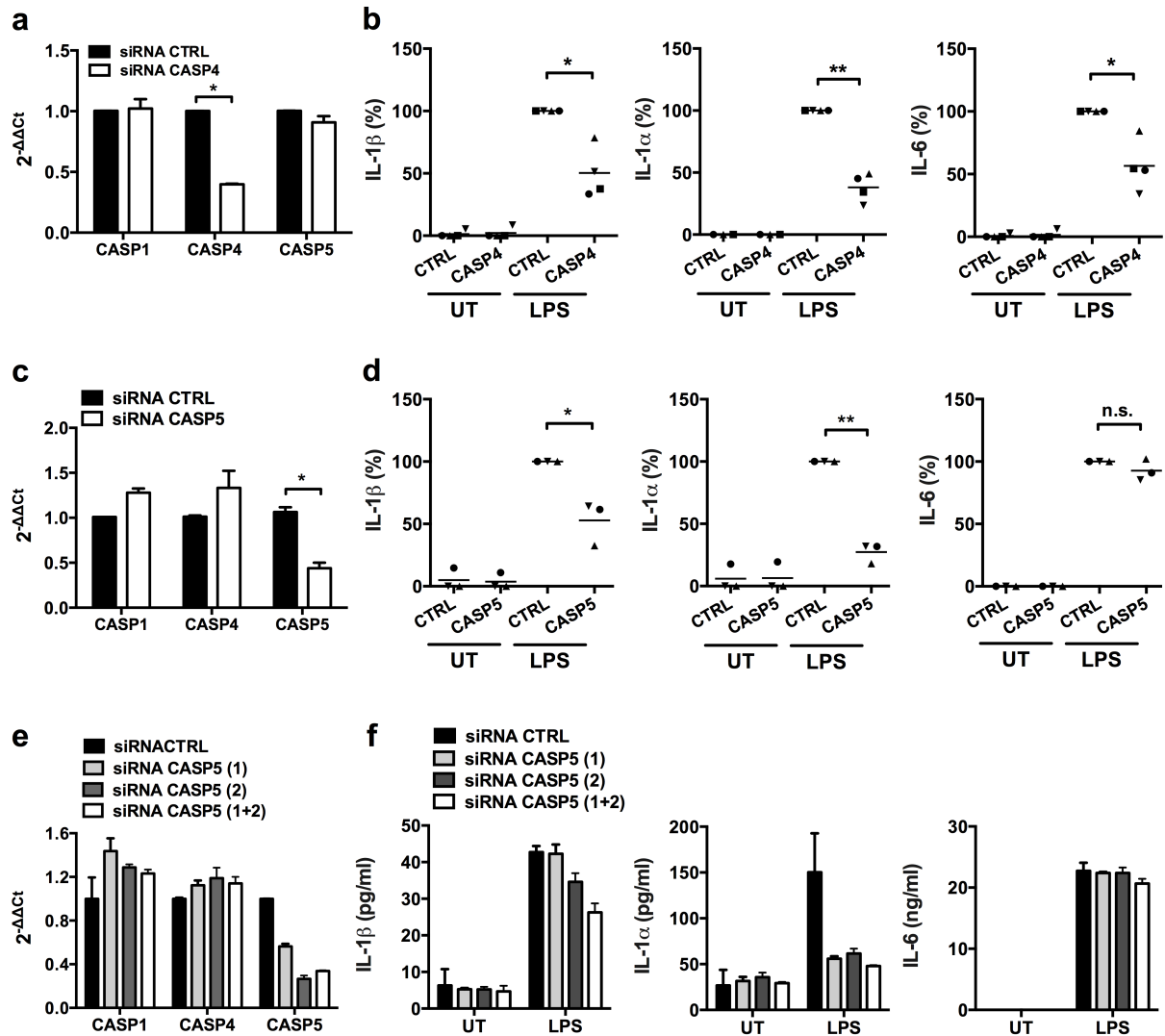


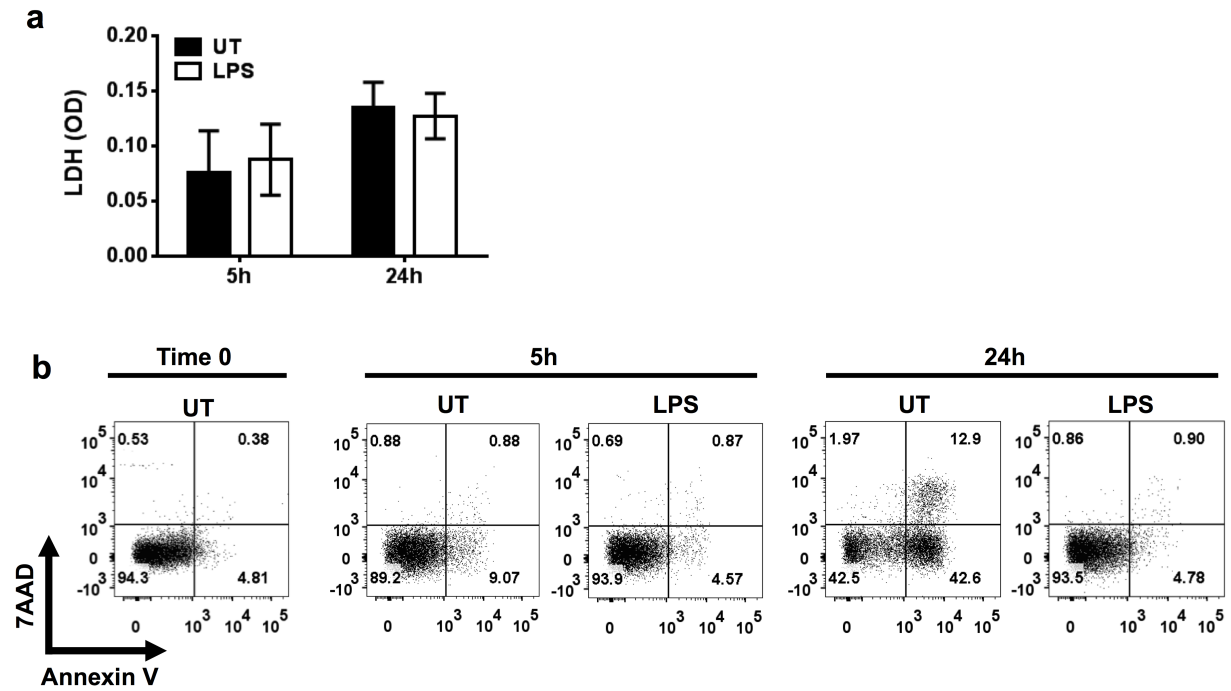
SUPPLEMENTARY FIGURES



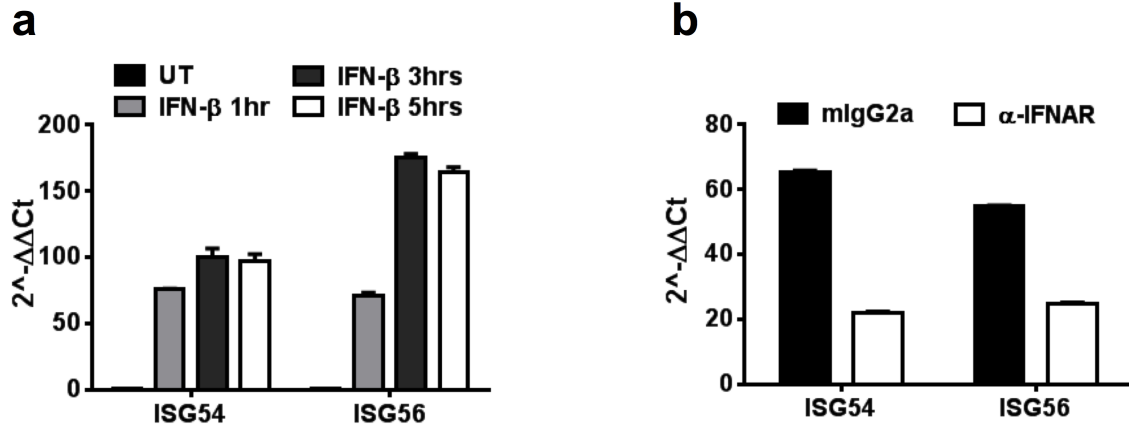
Supplementary Figure 1. Dendritic cells and macrophages require a second stimulus to activate the NLRP3 inflammasome. ELISA (a,c) and western blot (b,d) analyses of dendritic cells (a,b) and macrophages (c,d) cultured in medium alone, or stimulated with LPS in the presence or absence of ATP. Secretion of IL-1 β and IL-6 cytokines (a,c) was determined in supernatants, and pro-IL-1 β , pro-caspase-1, and caspase-1 p20 expression levels (b,d) in cell lysates. GAPDH was used as the loading control. Graphs show the mean \pm standard deviation of triplicate wells and are representative of three independent experiments.



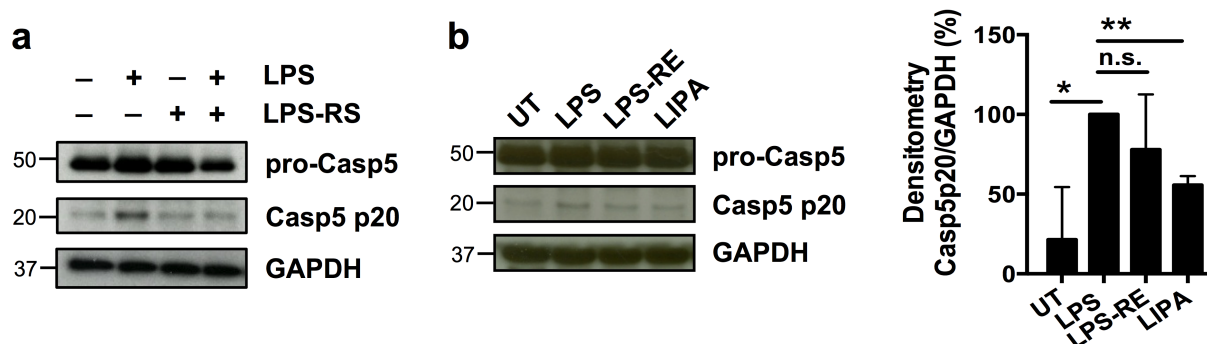
Supplementary Figure 2. Caspase-4 and caspase-5 regulate the release of IL-1 β and IL-1 α from monocytes after LPS treatment. (a,c,e) Quantitative PCR analysis of caspase-1, -4 and -5 expression determined in monocytes after nucleofection of caspase-4 (a) or caspase-5 (c,e) siRNA duplexes. (b,d,f) IL-1 β , IL-1 α and IL-6 release from unstimulated and LPS-stimulated monocytes in which caspase-4 (b) or caspase-5 expression (d,f) was knocked down. Graphs show the mean \pm standard deviations normalized as percent of the LPS-stimulated siRNA control. Each value represents monocyte response of independent donors and significance evaluated using one sample t-test. * p-value < 0.05, ** p-value < 0.01.



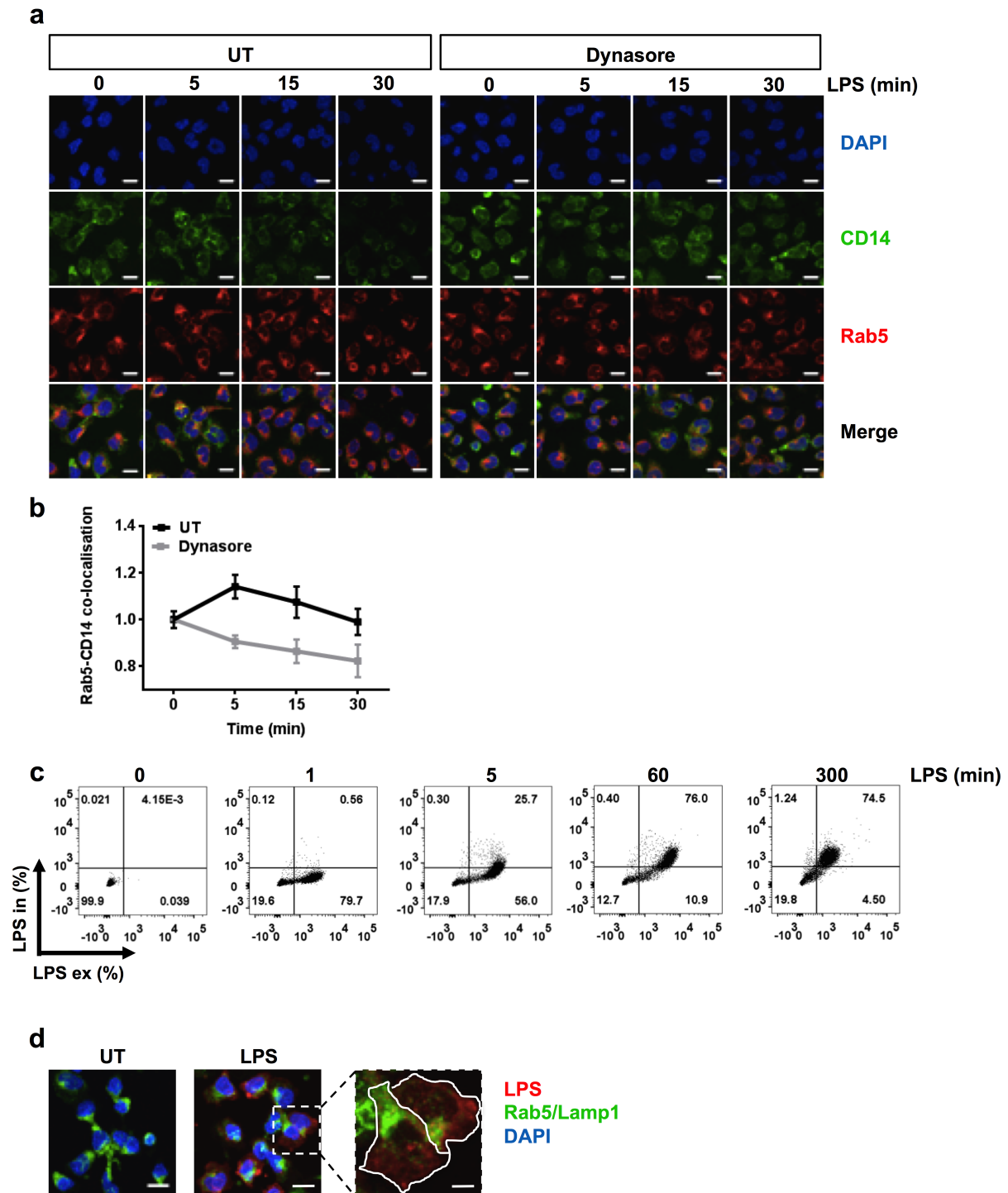
Supplementary Figure 3. Human monocytes do not undergo cell death following LPS stimulation. Cytotoxicity was determined by LDH released in cell-free supernatants (a) or as annexin V/7-AAD staining (b) of monocytes left untreated or stimulated with LPS for 5 and 24h. Graph a shows the mean \pm standard deviation of three independent experiments.



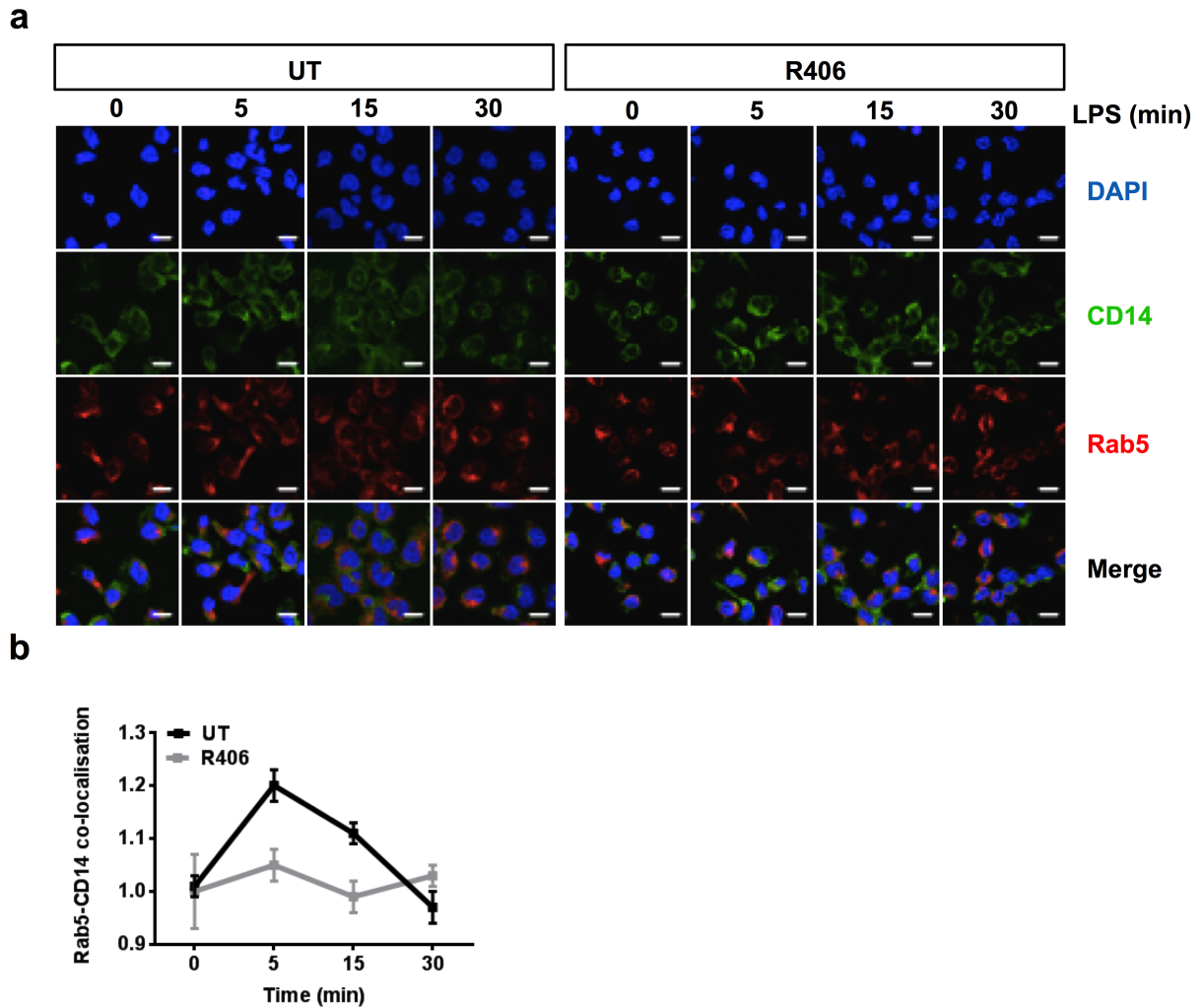
Supplementary Figure 4. Effect of IFNAR blocking on expression of IFN stimulated genes. ISG54 and ISG56 expression were assessed by quantitative real-time PCR in monocytes treated with IFN-β for the indicated times (a) or in monocytes that were pre-treated with a neutralizing anti-IFNAR antibody (α-IFNAR) or isotype-matched control (mIgG2a) prior to IFN-β stimulation (b). Graphs show the mean ± standard deviation of triplicate wells and are representative of three independent experiments.



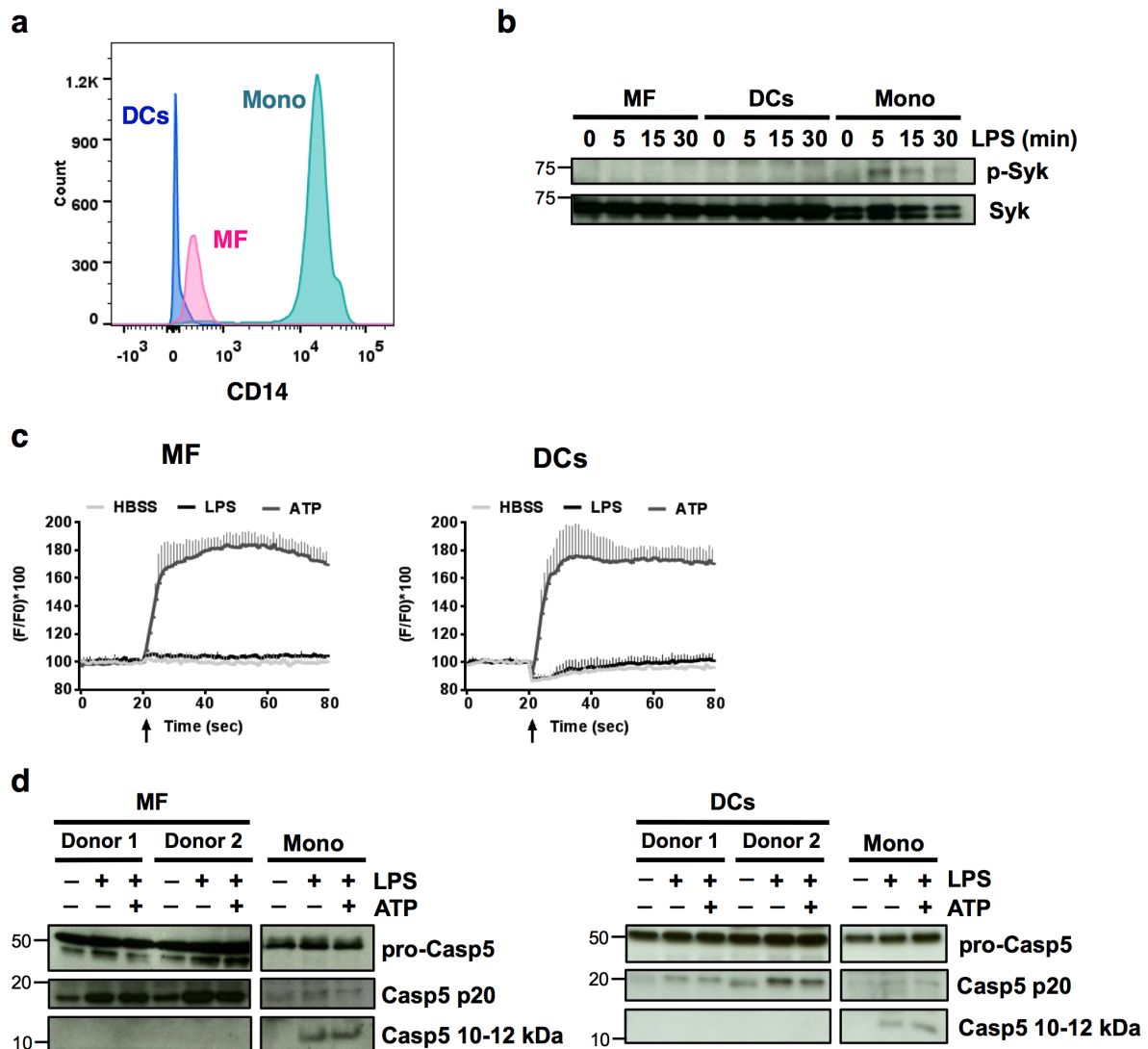
Supplementary Figure 5. Caspase-5 processing requires TLR4 signaling. (a) Monocytes were treated with the LPS antagonist LPS-RS from *Rhodobacter sphaeroides* (10 µg/ml) for 1h prior to LPS stimulation. Caspase-5 processing was measured in lysates by western blot. (b) Caspase-5 processing in monocytes stimulated with LPS, LPS-RE or lipid A (LIPA) was assessed by western blot (left). Densitometry analysis was performed and caspase-5 p20 expression is shown on the right as mean \pm standard deviation of three independent experiments and significance evaluated using a one sample t-test. * p-value < 0.05, ** p-value < 0.01, n.s. not significant.



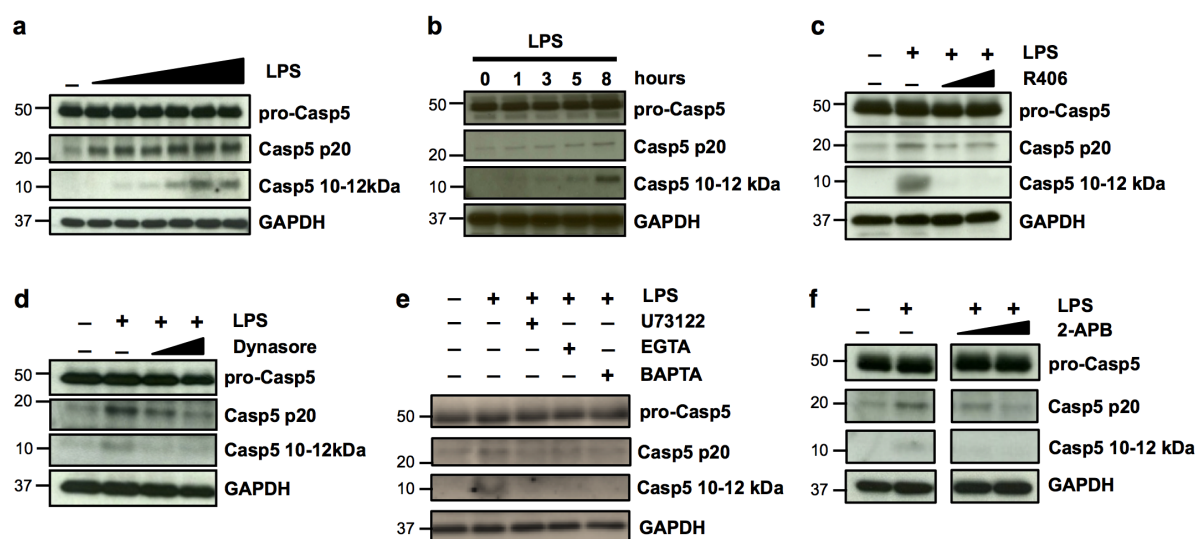
Supplementary Figure 6. Endosomal re-localization of CD14 in LPS-stimulated monocytes. (a,b) CD14/Rab5 co-localization in monocytes that were treated or not with dynasore prior to LPS stimulation. Co-localization was evaluated using images from three independent experiments for each time point, and Pearson's correlation coefficient was calculated by averaging values from ~100 cells across five imaging fields. Scale bar, 10 μ m. (c) Membrane-bound LPS (LPS ex) and intracellular LPS (LPS in) were determined by flow-cytometry analysis of monocytes that were treated or not with biotinylated LPS for the indicated times. Inset numbers indicate the proportion of positive cells in each quadrant. (d) Z-stack projections of the focal planes of monocytes that were untreated (UT) or stimulated with LPS for 1h (LPS in red, Rab5/Lamp1 early and late endocytic markers in green, and nuclear staining in blue). Scale bar, 10 μ m.



Supplementary Figure 7. Endosomal CD14 re-localization depends on Syk. (a) Co-localization of CD14 (green) and Rab5 (red) in monocytes that were pre-treated with the Syk inhibitor R406 for 30min and then stimulated with LPS for the indicated times. Nuclei were stained using DAPI (blue). Scale bar, 10 μ m. (b) Co-localization was evaluated using images from three independent experiments for each time point, and Pearson's correlation coefficient was calculated by averaging values from ~100 cells across five imaging fields.



Supplementary Figure 8. Defective Syk activation and Ca^{2+} mobilization in macrophages and DCs following LPS stimulation. (a) CD14 expression in monocytes (mono), macrophages (MF) and dendritic cells (DCs) differentiated in culture from the same donor. (b) Syk phosphorylation was assessed in MF, DCs and monocytes upon LPS stimulation for 5, 15 and 30min by western blot analysis. (c) Mobilization of Ca^{2+} in MF (left) and DCs (right) after stimulation with LPS (1 $\mu\text{g}/\text{ml}$) or ATP (1 mM). Arrows indicate the timing of stimuli addition. (d) Caspase-5 processing in MF (left), DCs (right) and monocytes stimulated with LPS, LPS/ATP or left untreated. GAPDH was used as the loading control. Graphs show the mean \pm standard deviation of triplicate wells and are representative of three independent experiments.



Supplementary Figure 9. Identification of a caspase-5 10-12 kDa fragment associated with LPS response in monocytes. Western blot analyses of caspase-5 processed forms (precursor, p20 subunit and 10-12 kDa) in lysates from monocytes stimulated with different LPS doses (a) and LPS exposure times (b), or in lysates of monocytes pre-treated with Syk inhibitor (c), dynamin inhibitor (d), the PLC γ inhibitor U73122 (e), the Ca²⁺ chelators EGTA and BAPTA (e), or IP3 receptor antagonist (f). GAPDH was used as the loading control. The blots shown here are the same as those shown in Fig. 2d, 2e, 5c, 5d, 7b and 7c.

a

1 MAEDSGKKKR RKNFEAMFKG ILQSGLDNFV INHMLKNNVA GQTSIQLTLP NTDQKSTSVK

61 KDNHKKKTVK MLEYLGKDV L HGVFNYLAKH DVLTLKEEEK KKYD TKIED KALILVDSL R

121 KNRVAHQMFT QTLLNM **DQKI** **TSVKPLLQIE** **AGPPESAEST** **NILKLCFREE** **FLRLCKKNHD**

181 **EIYPIKKRED** **RRRLALIEN** **TKFDHLPARN** **GAHYDIVGMK** **RLQGLGYTV** **VDEKNLTAR** **D***

241 **MESVLR** **AFAA** **RPEHKSSDST** **FLVLMHGIL** **EGICGTAHKK** **KKPDVLLYDT** **IFQIFNNRNC**

301 **LSLKDKPKVI** **IVQACRGEKH** **GELWVRD** SPA SLALISSQSS ENLEA **DSVCK** **IHEEKDFIAF**

361 **CSSTPHNVSW** **RDRTRGSIFI** **TELITCFQKY** **SCCCHLMEIF** **RKVQKSFEVP** **QAKAQMP TIE**

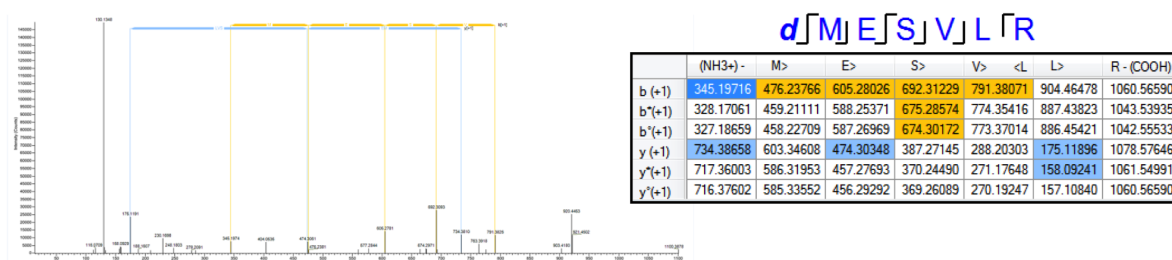
421 **RATLTRDFYL** **FPGN**

b

Sequence: DMESVLR

D1-TMT6plex (229.16293 Da)

Charge: +2, Monoisotopic m/z: 539.79230 Da (+0.43mmu/+0.79 ppm), MH⁺: 1078.57732 Da



Supplementary Figure 10. Alternative proteolytic cleavage of caspase-5. (a) Amino acid sequence of human caspase-5 isoform a. Caspase-5 enzyme is synthesized as a 48 kDa precursor polypeptide, which contains a pro-domain (in black) followed by the 20 kDa subunit (p20, in ochre) joined by a short linker peptide (in black) to the 10 kDa subunit (p10, in blue). An alternative Asp (D)-X cleavage site within the p20 subunit has been identified (D*240). DMESVLR peptide (in green) corresponds to the N-terminus labeled peptide detected by mass spectrometry. L146 (in red) and the surrounded area correspond to the epitope recognized by the anti-caspase-5 antibody. The caspase-5 active site (C315) is highlighted in purple. (b) MS/MS spectrum of peptide DMESVLR (2+) from caspase-5 was extracted and ion series (b, y) were annotated using PD 1.4 with clear N-terminal modification (TMT-reporter ion 130.1348 m/z). Detailed ion masses are shown in the table next to the spectrum.

Fig 1b

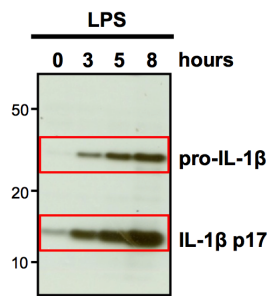


Fig 1c

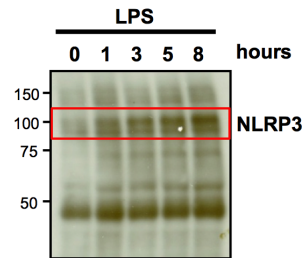


Fig 1d

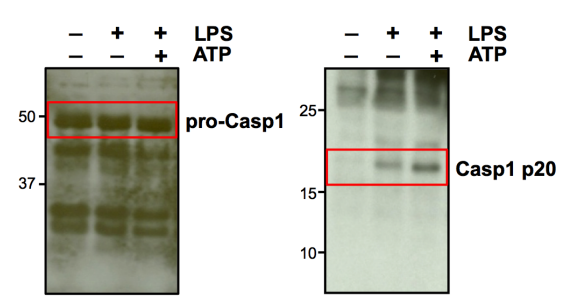


Fig 1g

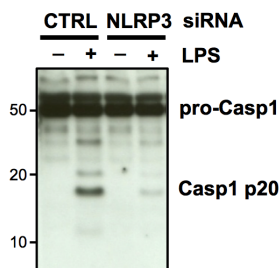


Fig 2c

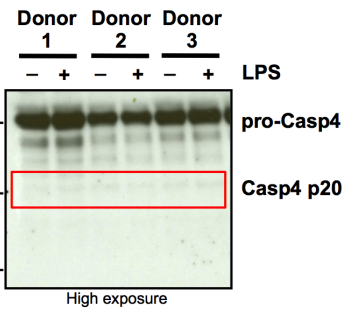
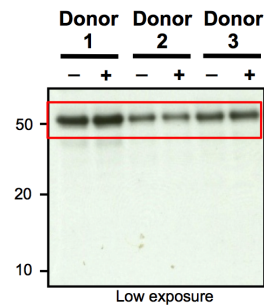


Fig 2d & Suppl. Fig 9a

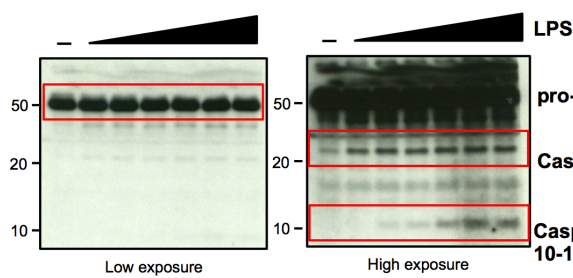


Fig 2e & Suppl. Fig 9b

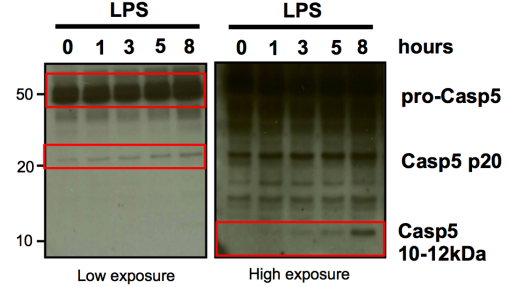
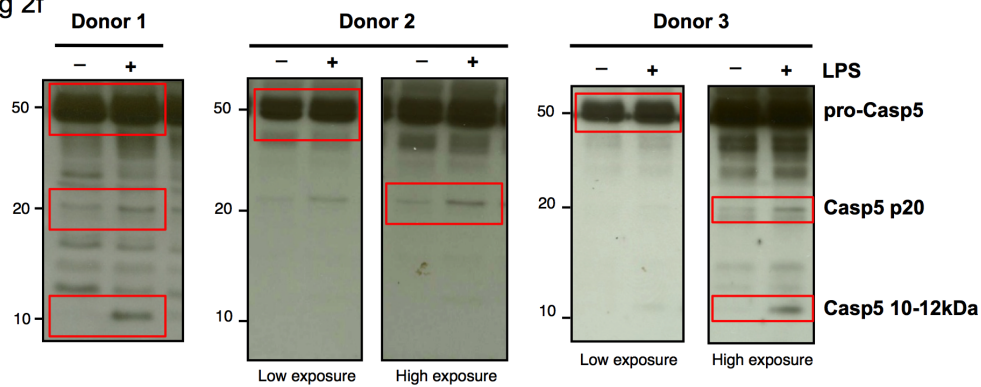


Fig 2f



Cont'd

Fig 3b

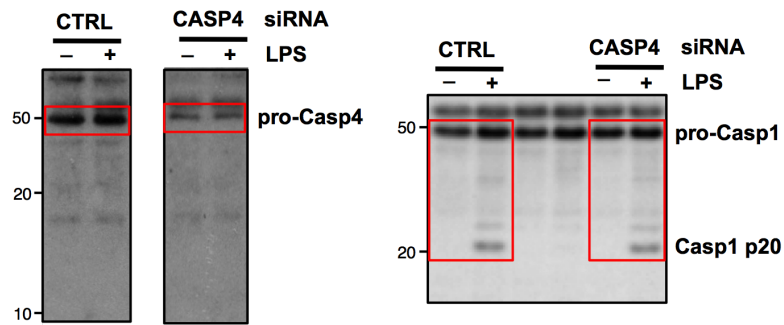


Fig 3d

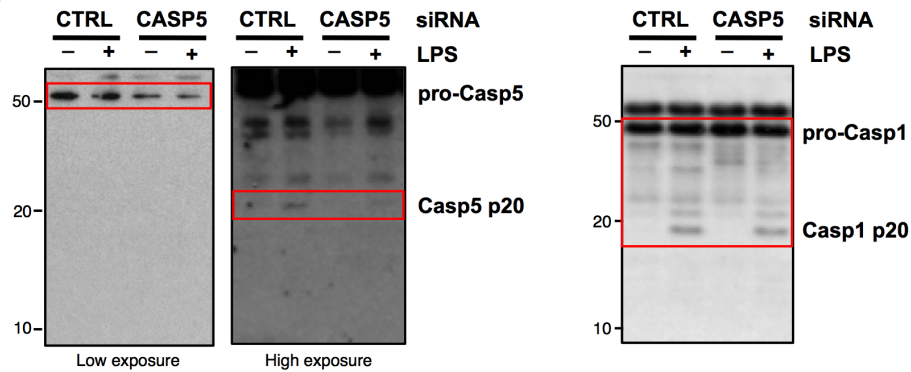


Fig 4b

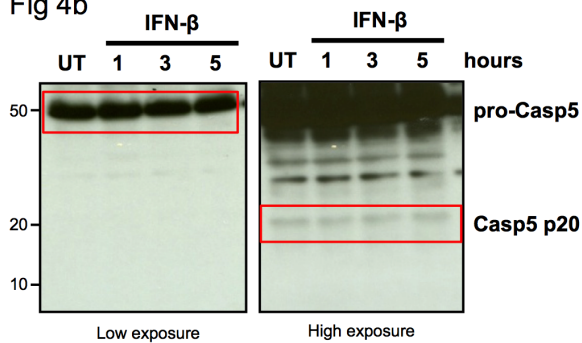


Fig 4d

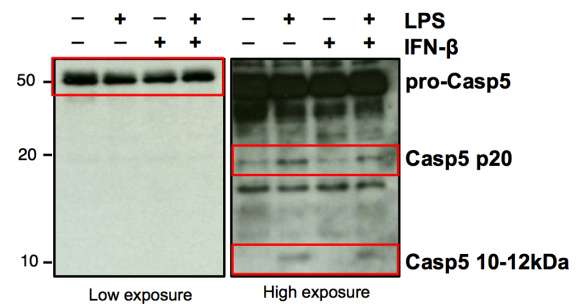
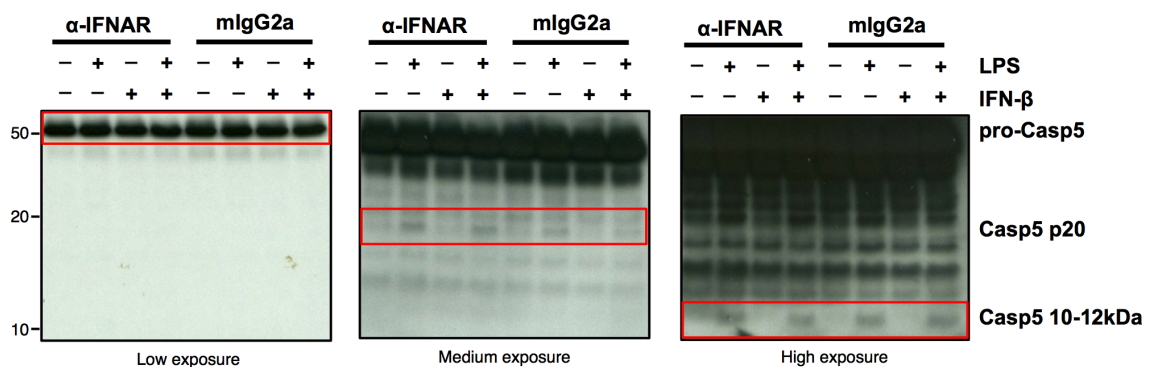


Fig 4f



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Fig 5a

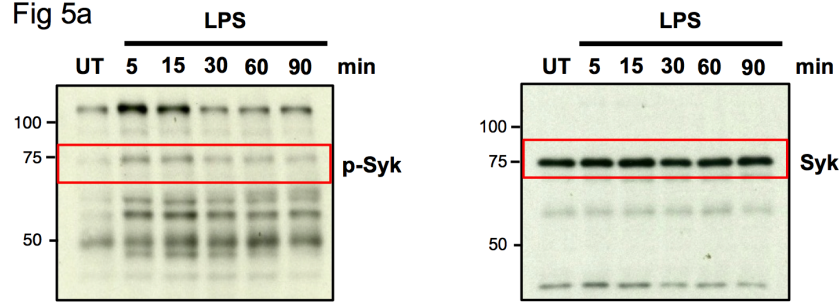


Fig 5b

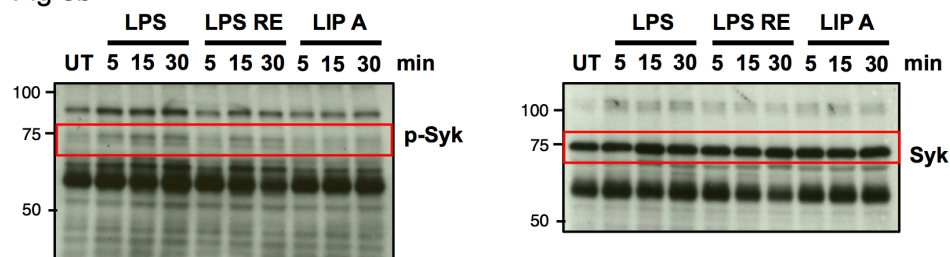


Fig 5c & Suppl. Fig 9c

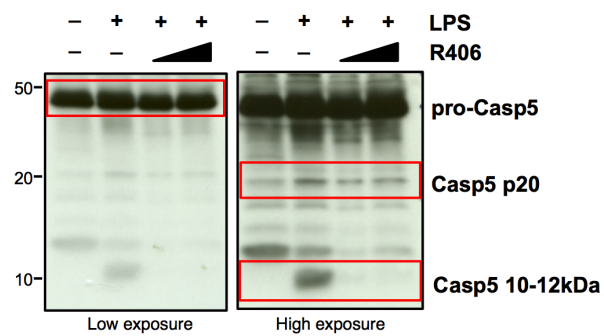


Fig 5d & Suppl. Fig 9d

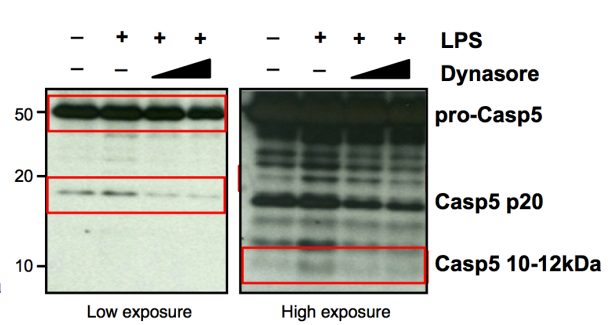
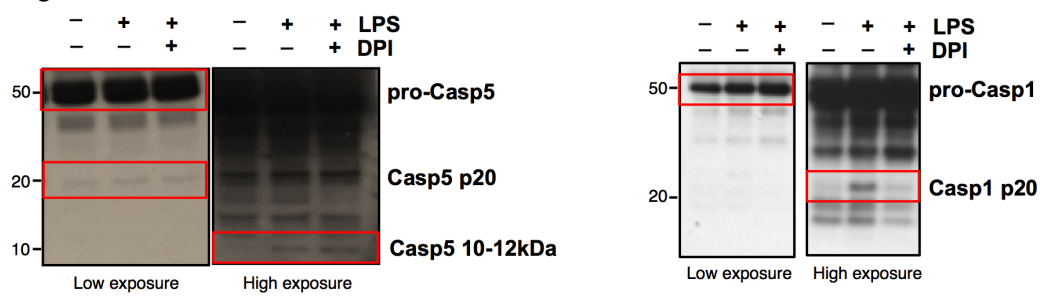


Fig 6b



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Fig 7b & Suppl. Fig 9e

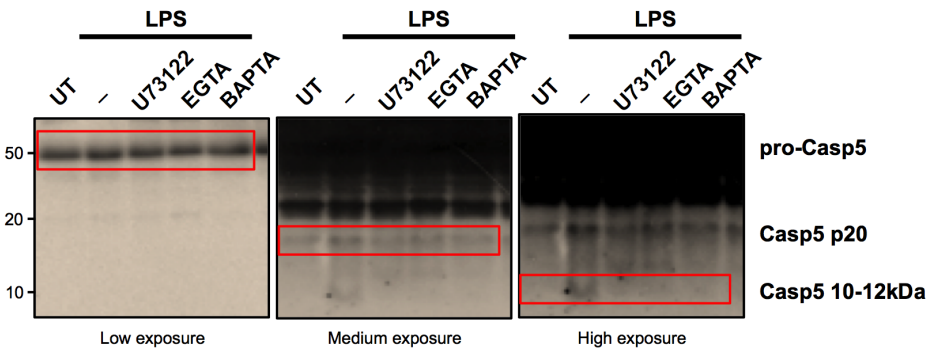
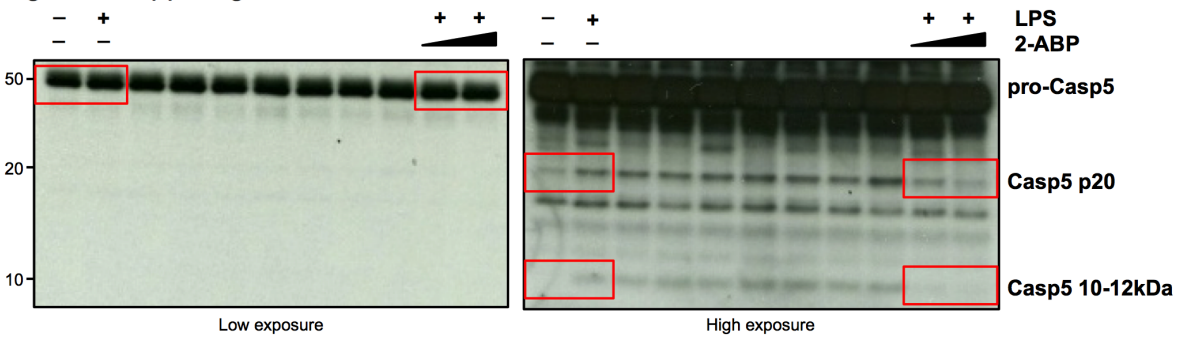
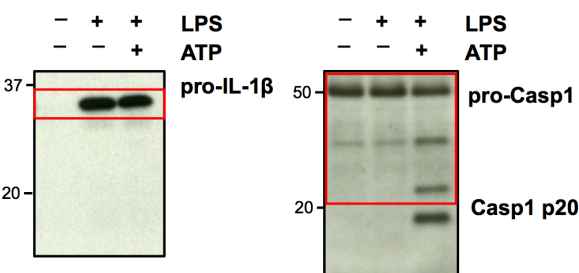


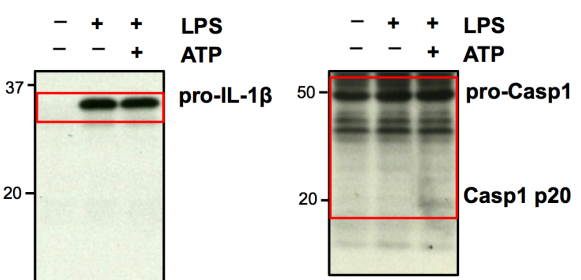
Fig 7c & Suppl. Fig 9f



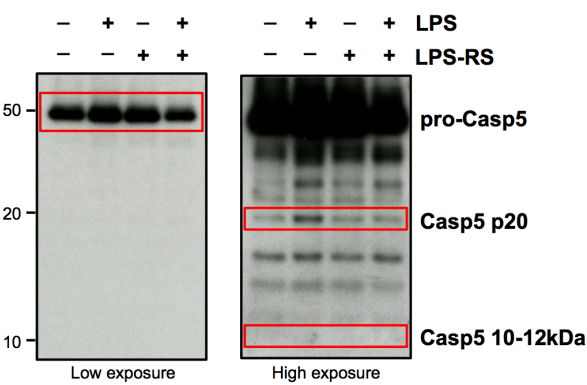
Suppl. Fig 1b



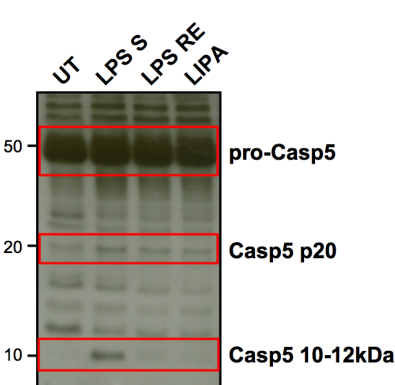
Suppl. Fig 1d



Suppl. Fig 5a

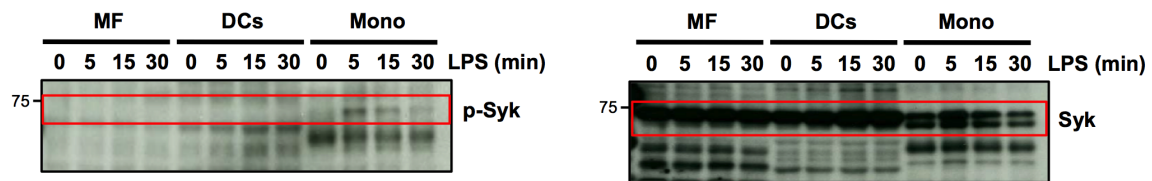


Suppl. Fig 5b

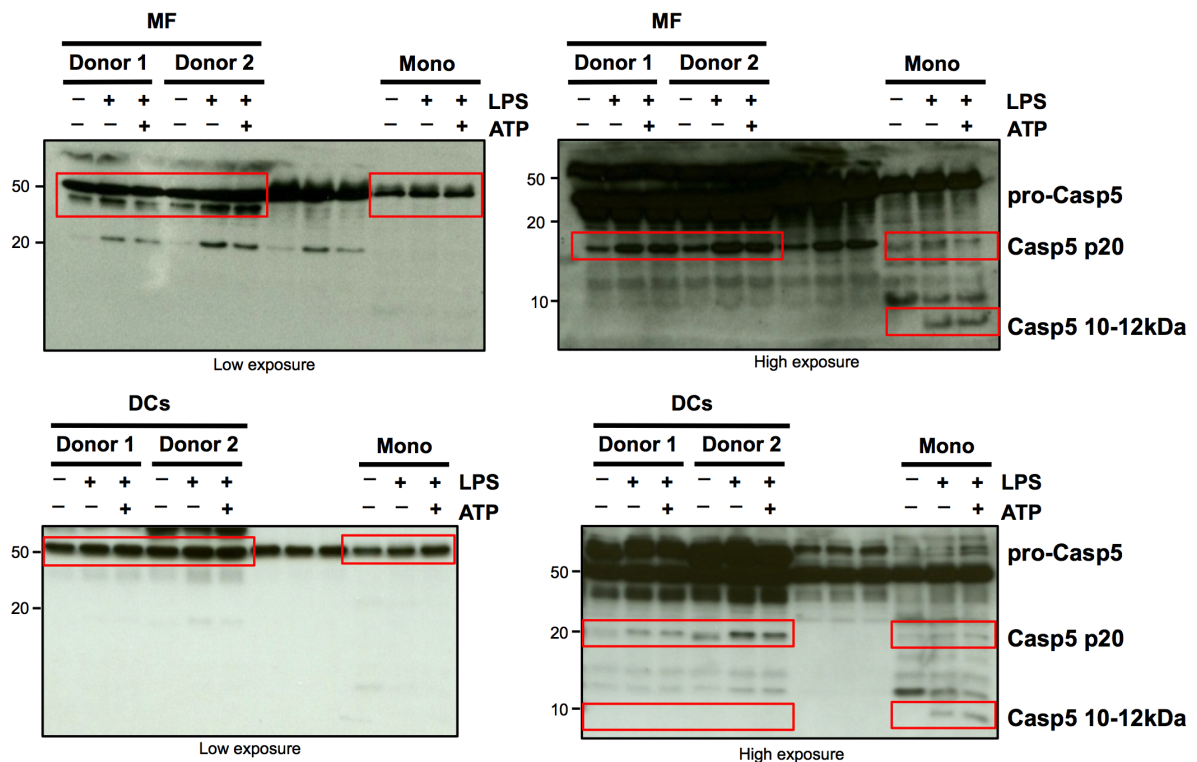


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Suppl. Fig 8b



Suppl. Fig 8d



Supplementary Figure 11. Uncropped blots related to the western blots shown in Figures 1-7 and Supplementary Figures 1, 5, 8 and 9. The red boxes highlight the regions of the western blots shown in the figures.